Localization and function of GABA transporter 1 in the retina

Giovanni Casini

Dipartimento di Scienze Ambientali, Università della Tuscia, I-01100 Viterbo, Italy

Running title: GAT-1 in the retina

Number of text pages: 23

Number of figures: 7

Number of tables: 1

Address for correspondence:
Giovanni Casini, Ph.D.
Dipartimento di Scienze Ambientali
Università della Tuscia
Largo dell'Università snc, Blocco D
01100 Viterbo
Italy

Tel: +39 0761 357 040
Fax: +39 0761 357 179
e-mail: gcasini@unitus.it

Summary

Plasma membrane transporters, located in the presynaptic terminal and/or surrounding glial cells, terminate synaptic transmission by operating rapid, high affinity uptake of the neurotransmitter from the synaptic cleft. Pharmacological blockade of transporters increases extracellular neurotransmitter levels and prolongs transmitter exposure to the receptors. GABA transporters (GATs) belong to the Na^+ and Cl^--dependent transporter family. Four GATs have been isolated and cloned in mammals. Of them, GAT-1 and GAT-3 are expressed in the retina. GAT-1 has a widespread distribution to different retinal cell types, but it is prominently expressed in amacrine cells of all vertebrate species studied to date. There are some species differences in the expression patterns of GAT-1 in the retina. It is expressed by horizontal cells in non-mammalian but not in mammalian retinas, and it is expressed in Müller glial cells of rats and guinea pigs, but not of rabbits and primates.

Functionally, GAT-1, together with GAT-3, regulates the extracellular GABA levels in the retina, thereby determining the level of inhibitory interactions and affecting visual processing in the retinal pathways. GAT-1 may interact with $GABA_C$ receptors on bipolar cell terminals and influence ganglion cell responses. It may also interact with $GABA_B$ receptors in the regulation of retinal waves of spontaneous activity which are known to play critical roles during development of the visual system. Other important functional actions are exerted by GAT-1 through reversed GABA transport. These include GABA release by cholinergic/GABAergic starburst amacrine cells and GABA release during early retinal development.

Keywords: horizontal cells, amacrine cells, ganglion cells, Müller cells, GABA receptors, GABA uptake, reversed transport, retinal development.

1. Neurotransmitter transporters

Chemical neurotransmission is a highly complex process which involves release of neurotransmitter from the presynaptic terminal, diffusion across the synaptic cleft, and binding to receptors on the postsynaptic membrane, resulting in a response of the postsynaptic neuron. Overall, neurotransmitter transporters can be classified as intracellular vesicular transporters, which are responsible for sequestering transmitters from the cytoplasm into synaptic vesicles, and plasma membrane transporters, located in the presynaptic terminal and/or surrounding glial cells, which terminate synaptic transmission by operating rapid, high affinity uptake of the neurotransmitter from the synaptic cleft (1). The transmembrane transport of neurotransmitters is of fundamental importance for proper signaling between neurons, as it exerts a key role in controlling the neurotransmitter concentration in the synaptic cleft.

Plasma membrane transporters have long been recognized as important components of the machinery for neural signaling. Reuptake inhibitors increase the levels of neurotransmitter in the synapse, thus enhancing synaptic transmission, and provide important targets for therapeutic intervention. Indeed, the importance of neurotransmitter transporters is highlighted by the broad spectrum of drugs targeting these proteins, including those used to treat depression, anxiety, obesity and epilepsy, in addition to drugs of abuse, such as cocaine, amphetamine and ‘ecstasy’ (2). Furthermore, it is well established that neurotransmitter transporters have roles in several neurological and psychiatric diseases, including amyotrophic lateral sclerosis, severe orthostatic hypotension, obsessive–compulsive disorder, Asperger’s syndrome, anorexia and autism (3).

Cloning studies show that two distinct gene families encode neurotransmitter transporters. One family codes for the high-affinity glutamate transporters (SLC1 gene family), the other codes for the Na^+ and Cl^--coupled transporters (SLC6 gene family). The latter subclass is the largest and includes transporters of dopamine, serotonin, norepinephrine, glycine and GABA (3). Additional proteins may associate with transporters in native systems. Indeed, transporters, similar to receptors and ion channels, are likely to be associated with other proteins that are necessary for their localization near the sites of transmitter release and/or for regulation of transporter function (4).

Important functions of plasma membrane transporters not only include the termination of the signaling process by neurotransmitter reuptake, but also replenishment of the neurotransmitter supply inside nerve terminals and, in the case of the glutamate transporters, keeping the extracellular concentration of glutamate below neurotoxic levels. In addition, following membrane depolarization, they can also work in reversed mode and mediate nonvesicular, Ca^{2+}-independent transmitter release from presynaptic terminals (5). Transmitter release by reversed transport can be modulated by other neurotransmitters. For instance, both GABA and glutamate may reciprocally influence their releases through plasma membrane transporters (6).

2. GABA transporters

In a GABAergic synapse, about 80% of the released GABA is likely to be transported back into the GABAergic nerve ending, while the remaining 20% is taken up by the astrocytes surrounding the synapse. GABA transporters (GATs) belong to the Na^+ and

Cl^--dependent transporter family, composed of several subfamilies including the choline, monoamine, taurine, glycine, and betaine amino acid transporters. Most of these proteins are made up of approximately 600 amino acid residues, and display a molecular mass of around 80 kDa. Some of these transporter subfamilies show a common structural organization, characterized by 12 transmembrane segments, organized in dimers. A typical conserved feature is the wide extracellular loop localized between segments 2 and 4. This structure acts as a site for glycosilation, important for the final stage of the insertion of transporters in the plasma membrane (7). These proteins also contain three sites of phosphorylation through protein kinase C, and one through protein kinase A, likely acting in the functional regulation of the transporter. Both the N- and C-terminal regions are located in the cytosol (8).

The mechanism of the Na^+ and Cl^--dependent transport is complex, and it depends on ion cotransport directed by electrochemical gradients. GABA transport is electrogenic with a stoichiometry for Na^+:Cl^-:GABA transport of 2:1:1 (all inwards). In the presence of membrane depolarization and of appropriate gradients, reversed transport of GABA may occur (5, 9). GATs are influenced by a variety of physiological stimuli, including GABA (10), brain derived neurotrophic factor (11) and hormones (12), and they are regulated by multiple intracellular effectors including protein kinase C and syntaxin 1A (4, 10, 11, 13).

To date, three GATs and a betaine glycine transporter (BGT), which also transports GABA with high affinity, have been isolated and cloned in mammals (14). Unfortunately, different nomenclatures have been used for the GAT subtypes in the mouse and in the rat and human, and the resulting picture is rather misleading. GAT1, GAT2, GAT3 and GAT4 in the mouse nomenclature correspond to GAT-1, BGT-1,

GAT-2 and GAT-3, respectively, in the rat and human nomenclatures (15). The rat/human nomenclature will be used in this chapter.

GAT-1 is considered a 'neuronal' transporter, while GAT-2 and GAT-3 are believed to be 'glial' transporters. The role of BGT-1 in GABA uptake in the brain is not well understood (16). GAT-1 is the most widely expressed GAT in the central nervous system, mainly localized to presynaptic axon terminals forming symmetric synaptic contacts, and to few astrocytic processes. GAT2 is weakly expressed throughout the brain, however it is primarily present in the leptomeninges and in ependymal and choroid plexus cells and only to a minor extent in neuronal and non-neuronal elements. GAT-3 is almost exclusively localized to distal astrocytic processes (14).

A number of inhibitors of GABA transport have been discovered (17). At least two high affinity systems were first identified based on the effects of the GABA uptake inhibitors cis-3-aminocyclohexanecarboxylic acid (ACHC) and β-alanine. In particular, ACHC, but not β-alanine, strongly inhibits GABA uptake by GAT-1. ACHC inhibition is considered a typical property of neuronal transporters. In contrast, GABA uptake by GAT-2 and GAT-3 is strongly inhibited by β-alanine, but not by ACHC, which is considered a property of glial transporters (14). BGT-1 is competitively inhibited by betaine (18). Nipecotic acid and guvacine, two additional inhibitors of GABA transport, served as lead structures for the synthesis of a series of selective, high-affinity GAT-1 inhibitors, such as tiagabine, which penetrate the blood–brain barrier (19). Some of the main characteristics of GATs are reported in Table 1.

3. The GABAergic system in the retina

GABA is the major inhibitory neurotransmitter in the retina. Inhibitory signaling pathways play a fundamental role in the shaping of visual information by modulating the flow of visual inputs from photoreceptors to bipolar cells and, subsequently, from bipolar cells to ganglion cells. The initial inhibitory interactions in the outer plexiform layer (OPL) are mediated by horizontal cells, while those in the inner plexiform layer (IPL) are mediated by amacrine cells (Fig. 1). Although the possibility of horizontal cell-cone photoreceptor interactions is plausible in mostly non-mammalian retinas (see (20) for review), generally GABA does not seem to contribute to lateral inhibitory interactions in the OPL (21-23). It is well established, instead, that GABA mediates the lateral inhibitory phenomena in the IPL, which contribute to the surround of ganglion cell receptive fields (24, 25). The GABA-mediated inhibition in the IPL may also be involved in the temporal responses of ganglion cells (26) and plays a fundamental role in ganglion cell motion and direction sensitivity (27). Recent papers have reviewed the localization patterns of GABA and its receptors as well as the physiology of the inhibitory GABAergic network in the vertebrate retina (20, 28-30).

GABA-containing retinal neurons are horizontal cells, amacrine cells, interplexiform cells (an amacrine cell variant), bipolar cells and ganglion cells. The issue of whether horizontal cells can be generally considered as GABAergic is destined to remain unresolved. Indeed, they appear to contain GABA or GAD in some vertebrate retinas but not in others, with high variability also depending on the developmental time and the retinal location (see (29) for review). Small subsets of bipolar cells may contain GABA in a number of vertebrate retinas. In primates, for instance, there are conflicting

reports of the presence or the absence of GABA immunoreactivity in bipolar cells (see (31) for references). GABA-containing amacrine cells constitute one of the largest neuronal populations in the retina, and they account for 37-38% of the total amacrine cells in the rabbit retina (32). A number of subpopulations of GABA-containing amacrine and interplexiform cells can be identified on the basis of coexpressed transmitters/peptides (see (33, 34) for references). In the ganglion cell layer (GCL), both displaced amacrine cells and ganglion cells containing GABA have been reported (35).

Three types of GABA receptors, with distinct structural and pharmacological characteristics, mediate inhibition in the retina: the ionotropic $GABA_A$ and $GABA_C$ receptors, and the metabotropic $GABA_B$ receptors (see (20) for review). Activation of these receptors leads to neuron hyperpolarization, with subsequent reduction of transmitter release and/or action potential firing. $GABA_A$ receptors are pentameric and consist of α, β, γ, and δ subunits. The pharmacology of these receptors may vary depending on the assembled subunits. The most common combination is α1/β2/γ2, which is widely expressed in the central nervous system, including the retina. These receptors are directly coupled with Cl^- channels, can be specifically blocked by bicuculline and have modulatory binding sites for benzodiazepines, barbiturates, ethanol and neurosteroids. $GABA_B$ receptors are G-protein-coupled receptors, with second messenger pathways leading to increase in K^+ conductance, or decrease in voltage-dependent Ca^{2+} currents. The $GABA_B$ receptor is activated by baclofen and it is insensitive to bicuculline. $GABA_C$ receptors are found predominantly in the retina. They are Cl^- pores, blocked by picrotoxin but insensitive to bicuculline and baclofen. Structurally, $GABA_C$ receptors characteristically consist of ρ subunits, which form

homoligomeric channels with $GABA_C$ receptor pharmacology. All of these receptors are widely expressed in vertebrate retinas (20, 28).

4. GABA uptake and GABA transporters in the retina

High affinity GABA uptake systems in the retina mediate GABA accumulation into neurons, Müller cells and retinal pigment epithelium cells with complex kinetic and pharmacological properties (36). In different vertebrate retinas, the uptake of GABA or GABA analogs has been extensively used in the past to map the retinal GABAergic system (37-43). In rabbit retina, GABA, the GABA analog diaminobutyric acid (DABA), the GABA agonist isoguvacine and the GABA uptake blocker nipecotic acid are taken up into neurons and Müller cells, while the GABA analogs γ-vinyl GABA and gabaculine are selectively accumulated, respectively, in amacrine cells and Müller cells (44). These uptake patterns suggest that multiple GABA transporters operate in the retina. They do not seem to operate in horizontal cells of rabbit and rat retinas, as it has been demonstrated that these cells do not take up GABA or GABA analogs (42, 44-48).

A variety of studies has established that there is a prominent expression of GATs in the retina. GAT-1, GAT-2 and GAT-3 mRNAs have been detected in rat retina using RT-PCR and Northern blot analysis (49, 50). Furthermore, they have been reported in developing and adult rat optic nerve by Northern blotting (51). GAT-1 is likely to be the principal GABA transporter in the retina (see below). GAT-2 do not seem to be expressed by cells of the neural retina. Indeed, GAT-2 immunoreactivity is predominantly distributed to the retinal pigment and ciliary epithelia (52-54), congruent with findings that cultured bovine retinal pigment epithelia accumulate GABA (55).

GAT-3 mRNA has been detected mostly in the INL of rat retina by in situ hybridization (56). Immunocytochemical analyses showed that it is distributed to all retinal layers, with an expression pattern indicating that it is predominantly localized to Müller cells in rat, rabbit and guinea pig retinas (52-54, 57). In addition, GAT-3 is also expressed by a number of amacrine and displaced amacrine cells originating a dense plexus of GAT-3 immunoreactive fibers in the IPL (53, 54, 57).

5. Localization of GAT-1 in the retina

After the cloning of GAT-1 from the rat brain (58), its expression in the rat retina, together with GAT-2 and GAT-3 expression, was first demonstrated with polymerase chain reaction analysis in 1992 (49). Subsequently, the presence of GAT-1 in retinas of other vertebrates has been investigated. For instance, a skate GABA transporter displaying high homology with the mouse GAT-1 has been detected by Northern blot in the skate retina (59), and GAT-1 immunoreactivity has been observed by immunoblot in goldfish retina (Klooster et al., 2004). In addition, studies of the uptake and release of GABA in the presence of the GAT-1 inhibitors nipecotic acid or NNC-711 provided evidence of the presence in chicken dissociated retinal cells in culture of a transporter with pharmacology similar, although not identical, to GAT-1 (60). The expression patterns of GAT-1 in the retina have been investigated with a variety of techniques and the presence of this transporter in selected retinal cell types has been reported.

5.1. GAT-1 in ganglion cells and/or displaced amacrine cells

The GCL contains both ganglion cells and displaced amacrine cells. The ganglion cells are the output neurons of the retina, and with their axons contact the primary visual nuclei in the brain. In contrast, the displaced amacrine cells are similar to the normally placed amacrine cells in the INL, their processes arborize in the IPL and they do not exit the retina (61). Using immunohistochemistry or in situ hybridization histochemistry, GAT-1 has been found to be expressed by cells in the GCL in tiger salamander (62), salmon (63), rat (50, 53, 56, 64), mouse (65, 66), rabbit (67), bovine (68), and primate (31) retinas. In most cases, these cells displaying GAT-1 immunoreactivity or GAT-1 mRNA have been interpreted as displaced amacrine cells mainly on the basis of the small soma size suggesting these cells were unlikely to be ganglion cells. However, retrograde transport of Fluorogold from the superior collicuclus combined with situ hybridization histochemistry demonstrated that at least a few of the GAT-1 mRNA expressing cells in the GCL of the rat retina are indeed ganglion cells (50). In addition, the observation of some GAT-1 immunostained fibers in the nerve fiber layer (NFL, which contains the ganglion cell axons) of the rabbit retina could indicate the presence of some GAT-1 expressing ganglion cells in the rabbit retina as well (67). This interpretation is not supported by observations in primate retinas, where GAT-1 immunopositive fibers have been observed in the NFL, but they did not reach the optic nerve, suggesting they are not ganglion cell axons (31). Furthermore, in mouse retina retrograde tracing from the superior collicuclus fails to label neuropeptide Y (NPY)-containing cells in the GCL, which also express GAT-1, indicating these cells are displaced amacrine cells (66).

Taken together, these data are consistent with the presence of GAT-1 in displaced amacrine cells, although a small number of ganglion cells may also express this transporter. The possibility also exists that a higher number of ganglion cells possess GAT-1. For instance, in primates GABA-immunoreactive ganglion cells and ganglion cell axons have been identified (69-72), but GAT-1-immunoreactive ganglion cells are unlikely (31). These GABA-containing ganglion cells, if they express GAT-1, are therefore likely to express it at their preterminal axonal processes and axonal terminals in retinal recipient nuclei, similar to the expression of GAT-1 on axonal terminals of hippocampal and cortical neurons (73, 74).

5.2. GAT-1 in amacrine cells

The majority of GAT-1-expressing cells in the retina are amacrine cells. The presence of GAT-1 in amacrine cells has been documented in most of the species studied to date (31, 50, 52-54, 56, 57, 62-68, 75-77), while in skate and bullfrog retinas strong GAT-1 immunostaining is reported in the IPL (78, 79), which suggests the presence of GAT-1-expressing amacrine cells. With immunohistochemical labeling, the IPL appears heavily GAT-1 immunostained, displaying intensely stained puncta and varicose processes. In some instances, GAT-1-immunolabeled somata have been reported within the IPL (interstitial amacrine cells) (31, 50). GAT-1-immunostained somata in the INL have often been observed to originate a process directed towards the OPL (31, 53, 54, 62, 67), where a meshwork of GAT-1-immunoreactive fibers has been detected in the monkey (31) and in the rabbit (Casini G., Rickman D.W. and Brecha N.C., unpublished) retina. The cells originating these processes are known as interplexiform cells, which can be considered an amacrine cell variant. The typical pattern of GAT-1

expression is represented in figure 2, which depicts an immunolabeling of the mouse retina (G. Casini, unpublished).

The predominant localization of GAT-1 to amacrine and interplexiform cells is generally congruent with the pattern of GABA- or GAD-containing cells and of GABA- or GABA analog-accumulating cells. Double label immunohistochemical studies have addressed the presence of GABA in GAT-1-expressing amacrine cells (Fig. 3). Colocalization of the two markers has been observed in amacrine cells of the goldfish retina (77), and quantitative data have been obtained in rabbit and in primate retinas. In one study of the rabbit retina, 99% of the GAT-1-immunoreactive amacrine cells were found to also contain GABA immunoreactivity, while 75% of the GABA-containing amacrine cells also expressed GAT-1 (67). Our unpublished observations in rabbit retina give an estimate of 66% of GABA-immunostained amacrine cells also displaying GAT-1 immunoreactivity (Casini G., Rickman D.W. and Brecha N.C., unpublished). Similar percentages (99% of GAT-1 amacrine cells also containing GABA and 66% of GABA-containing amacrine cells also displaying GAT-1) have been calculated in primate retinas (31). Since about 38% of all amacrine cells in rabbit retinas contain GABA (32), it follows that GAT-1 is expressed by 25-30% of all amacrine cells in these retinas. GABA-containing cells that do not express GAT-1 may take up GABA via another GAT, such as GAT-3, or they may lack a high-affinity GABA uptake system, similar to Purkinje cells of the cerebellum which do not express either GAT-1 or GAT-3 (80).

Interestingly, some GAT-1-expressing amacrine cells that do not contain GABA immunoreactivity have been reported in the rabbit retina (57). In particular, our quantitative studies of the rabbit retina (Casini G., Rickman D.W. and Brecha N.C.,

unpublished) show that this subset of GAT-1 / no-GABA cells amounts to 6.7% of all GAT-1-expressing amacrine cells, and a portion of them is likely to be represented by glycinergic AII amacrine cells (Fig. 4). The presence of GAT-1 immunoreactivity in cells that do not contain GABA is consistent with other reports that GAT-1 mRNA and immunoreactivity are also localized to non-GABA immunoreactive cells in other parts of the nervous system (81). The expression of GAT-1 by a small percentage of AII amacrine cells may be the consequence of errors during retinal formation. Indeed, during the early part of postnatal retinal development, about 32% of the amacrine cells contain both GABA and glycine immunoreactivity (82), and it is plausible that a small number of the cells destined to become glycinergic may maintain some GABAergic, non-functional characteristics.

Multiple subpopulations of GABA-containing amacrine cells have been detected in colocalization studies (33, 34), and the presence of GAT-1 in some of these subpopulations has also been tested. In the mouse retina, GAT-1 is expressed by the NPY-containing amacrine and displaced amacrine cells (66), while in monkey retina it is in vasoactive intestinal peptide (VIP)-containing amacrine cells (31). In contrast, GAT-1 immunoreactivity has not been reported in rabbit cholinergic amacrine cells (67) and in monkey dopaminergic amacrine cells (31). We have recently performed an extensive double-label quantitative immunohistochemical analysis of the composition of the population of GAT-1-expressing amacrine cells (Casini G., Rickman D.W. and Brecha N.C., unpublished). We found that the GAT-1-expressing amacrine cells include those containing VIP (Fig 5), those accumulating indoleamines and part of those containing substance P. In addition, about 20% of the GAT-1-immunoreactive amacrine cells also contain the calcium binding protein parvalbumin. GAT-1 immunostained

amacrine cells containing the calcium binding protein calbindin (CaBP) were also observed (Fig. 6). Together, these identified subgroups of amacrine cells account for 57% of all GAT-1 immunoreactive amacrine cells. The remaining 43% are likely to be composed of other GABA-containing amacrine cell populations (Fig. 7). Consistent with previous observations in rabbit and in monkey retinas (31, 67), we did not observe GAT-1 expression in cholinergic or in dopaminergic amacrine cells. The possibility exists that GAT-1 is expressed on the processes these cells and not, or very rarely, on their cell bodies, as it may be the case for some ganglion cells (see paragraph 5.1).

5.3. GAT-1 in bipolar cells

GAT-1-expressing bipolar cells have been demonstrated in tiger salamander retinas, where immunohistochemical studies have reported GAT-1 immunoreactivity in two different types of bipolar cells which also contained GABA (62). In addition, whole cell patch recordings from these cells revealed the presence of a GAT current (I_{GAT}) that was blocked by NO-711 (83), a selective GAT-1 antagonist (19). In contrast, bipolar cells expressing GAT-1 have not been reported in other vertebrate retinas. In particular, lack of colocalization of the immunoreactivities of GAT-1 and of a generic bipolar cell marker has been recently reported in the monkey retina (31).

5.4. GAT-1 in horizontal cells

In the retina of several vertebrate species, GABA is used by horizontal cells as a neurotransmitter (29). However, GAT-1 has not been localized to horizontal cells with in situ or immunohistochemical methods. For instance, horizontal cells identified by CaBP immunoreactivity in the rabbit retina are devoid of GAT-1 immunostaining

(Casini G., Rickman D.W. and Brecha N.C., unpublished, Fig. 6). Only in non-mammalian retinas there is electrophysiological and pharmacological evidence of electrogenic GABA transport mechanisms consistent with the presence of GAT-1 in horizontal cells. Indeed, I_{GATS} elicited by GABA uptake and/or release through a transporter have been detected in skate, catfish, goldfish, toad and tiger salamander horizontal cells, and these currents were sensitive to selective inhibitors of GABA transport through GAT-1, such as nipecotic acid, NO-711 or SKF89976A (84-92).

The lack of GAT-1 expression in mammalian horizontal cells is congruent with studies showing that these cells do not accumulate exogenous GABA or GABA analogs (42, 44-48), and that they also lack GAT-1 or GAT-3 expression (50, 53, 54, 57). However, horizontal cells in several mammalian species express GABA and GAD (93), suggesting that horizontal cells possess the machinery to synthesize and release GABA, but they do not have the cellular mechanisms to take up GABA from the extracellular space, similar to the Purkinje cell of the cerebellum (80). We may hypothesize that in these cases GABA released in the OPL could be transported from the extracellular space by GAT-1 located on processes of interplexiform cells or by GAT-3 expressed by Müller cells.

5.5. GAT-1 in Müller cells

A highly efficient GABA uptake system by Müller cells has been described in the guinea pig retina, where both GAT-1 and GAT-3 are expressed by Müller cells (54). In addition, the presence of GAT-1 in Müller cells of the rat retina has been demonstrated with in situ hybridization (50) and immunohistochemistry (53). GAT-1, however, is only weakly expressed by these cells, which instead show a prominent GAT-3

expression (50, 53). In non-mammalian retinas, both GAT-1 and GAT-3 have been immunohistochemically detected in Müller cells of the skate retina (78), while Müller cells of the bullfrog retina have been found to express GAT-1 and GAT-2 immunoreactivities, while they do not express GAT-3 (79).

5.6. Summary of the localization of GAT-1 in the retina

Taken together, the data presented above show that GAT-1 is abundantly expressed in vertebrate retinas. Overall, the distribution of GAT-1 in the retina is similar to the distribution of GABA and GAD immunoreactivities, and of GABA or GABA analogue uptake. GAT-1-expressing cells are mainly amacrine cells, including displaced and interstitial amacrine cells, and interplexiform cells. A few ganglion cells may also express this transporter, while it is not expressed by photoreceptors.

Different subpopulations of GAT-1-immunoreactive amacrine cells have been identified, and many GABA-containing amacrine cells that do not express GAT-1 have also been detected. These cells could express other GATs, such as GAT-3, or they could express GAT-1 on their processes but not on their cell bodies.

In mammalian retinas, GAT-1 is not expressed by horizontal or bipolar cells. The pattern of GAT-1 expression in mammalian retinas is consistent among mice, rats, guinea pigs, rabbits and primates, with the only exception that in rats and guinea pigs GAT-1 is weakly expressed in Müller cells, whereas it is not expressed in Müller cells of rabbits and primates.

The absence of GABA uptake systems in horizontal cells of mammalian retinas raises the question of how GABA can be removed from the outer retina. The possibility

exists that this function is operated by GAT-1 expressed on the distal processes of interplexiform cells and/or by GAT-1 and, mainly, GAT-3 expressed by Müller cells.

In non-mammalian retinas, similar patterns of GAT-1 distribution have been reported, with the notable exceptions of the presence of GAT-1 in horizontal cells and, at least in the case of the tiger salamander retina, in bipolar cells.

6. Functions of GAT-1 in the retina

6.1. Both GAT-1 and GAT-3 regulate GABA levels in the retina

The inhibition of GABA uptake mediated by GAT-1 in different regions of the central nervous system, including the retina, invariably results in increased tonic inhibition, suggesting that this transporter is a key player in the regulation of GABA neurotransmission. The widespread distribution of GAT-1 and GAT-3 in the retina indicates the presence of careful regulation of GABAergic signaling in the retina. For instance, GAT-1 and GAT-3 expressed by Müller cells would markedly influence GABA levels in all retinal regions, both by regulating GABA in the extracellular space and by limiting the spread of GABA from synapses (94). Moreover, the GATs expressed by Müller cells may protect the synapses from inappropriate inhibition by excess GABA released elsewhere during physiological and/or pathological events.

The characteristic morphology of Müller cells, which span the entire neural retina, enables these cells to influence GABAergic transmission both in the outer and in the inner retina, by taking up GABA released by horizontal cells and by amacrine cells, respectively. In contrast, GATs expressed by neurons are likely to both remove GABA from the synaptic cleft following depolarization and release GABA by reversed

transport. Indeed, there is good evidence for carrier-mediated release of GABA by non-mammalian horizontal cells (84, 86, 91) and by the acetylcholine- and GABA-containing starburst amacrine cells of the rabbit retina following depolarization (95).

In summary, GAT-1, expressed by neurons, is likely to be involved in GABA removal from the synaptic cleft and extracellular space and perhaps in GABA release by a Ca^{2+}-independent mechanism, thus modulating GABA levels and GABA actions at GABA receptors. GAT-1 and GAT-3 expressed by Müller cells could also influence the levels of GABA, acting both in the inner and in the outer retina, and probably limit the spread of GABA within the retina. GAT-1 and GAT-3 have different functional and pharmacological properties, including different ionic dependences and inhibitor sensitivities, that influence GABA uptake. This pharmacological and functional heterogeneity is likely to provide considerable flexibility in the control of the extracellular levels of GABA in different physiological states.

6.2. Regulation of GABA receptor activation by GAT-1 at retinal synapses

The transmission of the visual signal from bipolar cells to ganglion cells is influenced by $GABA_C$ receptors located on the bipolar cell axon terminals and by $GABA_A$ receptors located both on bipolar cell terminals and postsynaptic to the bipolar cell, on amacrine and ganglion cell dendrites (28). The activation of $GABA_C$ receptors importantly contributes to the regulation of bipolar cell output, and dysfunctions in $GABA_C$ receptor function result in altered ganglion cell responses (see (96) for references). The available evidence suggests that $GABA_C$ receptors may act to limit the extent of glutamate release from bipolar cell terminals. In the salamander retina, the selective GAT-1 blocker NO-711 determines an increase of the activation of $GABA_C$

receptors, with consequent reduction of the light-elicited signaling from bipolar cells to ganglion cells. This enhanced $GABA_C$-mediated response is likely to be due to activation of additional receptors by GABA spillover (97). The suppression of bipolar cell to ganglion cell transmission, provoked by GAT-1 blockade, affects the ganglion cell responses in a way similar to the reported effect of surround inhibition. These observations suggest that GAT-1 normally limits inhibitory signaling acting particularly at $GABA_C$ receptors. If GAT-1 were non-functional, additional $GABA_C$ receptors would be activated by spillover transmission, with consequent impairment of both the spatial and temporal properties of ganglion cell responses.

Recently, recordings from isolated bipolar cell terminals in goldfish retinal slices have shown that a tonic current mediated by $GABA_C$ receptors is maintained by spontaneous GABA release and that the level of this current is tightly regulated by GAT-1. In addition, GAT-1 selectively limits the $GABA_C$ receptor-mediated reciprocal feedback between bipolar cell terminals and amacrine cells (96). These observations implicate that GAT-1 is a major regulator of presynaptic excitability at the bipolar cell axonal terminal by setting the concentration of extracellular GABA in the vicinity of bipolar cell $GABA_C$ receptors. Supporting this conclusion, GAT-1 inhibition significantly increases the amount of current necessary to depolarize the bipolar cell terminal to its threshold for generating action potentials (96).

6.3. Effects of GAT-1 blockade on electroretinographic responses

In a recent study, the administration of the GAT-1 blocker NO-711 determined a pronounced increase of the b-wave of electroretinographic responses from isolated rabbit retinas (98). An explanation for this effect, proposed by the authors, is that as a

consequence of the reduced GABA uptake, amacrine cells would become depleted of their GABA content, and their release of GABA upon light stimulation would be reduced. Therefore, the inhibition mediated by GABA receptors on the ON-bipolar cell axonal terminals would also be reduced, and this would cause the increase of ON-bipolar cell activity and the enlargement of the b-wave, which can increase to double its normal size.

6.4. GAT-1 and retinal development

In adult retinas, GABA is predominantly expressed by amacrine cells. During development, GABA is transiently expressed in additional cells, including ganglion and horizontal cells. GABA uptake and release mechanisms and GABA receptors are also expressed early in retinal development, well in advance of the onset of visual function. In addition, GABA transporter mRNAs have been detected in the developing rat optic nerve (51). These observations suggest that the GABAergic system may serve a developmental role both in the establishment of retinal circuitries and in the organization of retinofugal projections (44, 99). The GABA transporter is likely to play extremely important roles, because it seems to be responsible for GABA release, through reversed transport, early in development, prior to the establishment of vesicular synaptic transmission (99).

Spontaneous activity during development is essential for proper differentiation and refinement of the nervous system. In the retina such activity takes the form of rhythmic waves of intracellular [Ca^{2+}] elevations (100). Recent observations have shown that endogenous GABA can modulate the frequency and the duration of such waves in the chick retina. This effect is mediated by $GABA_B$ receptors in a cAMP-independent

manner, and this action is influenced by GAT-1. In particular, it has been observed that the GAT-1 inhibitor SKF89976A reduces the frequency of the transients presumably because it induces an increase in the extracellular concentration of GABA (101).

6.5. Summary of the functions of GAT-1 in the retina

The experimental data reported in this section demonstrate that GABA transporters, and GAT-1 in particular, may affect multiple retinal functions, including important aspects of retinal development, by regulating extracellular levels of GABA. By modulating the availability of GABA in the extracellular milieu and/or at specific synapses, GAT-1 and GAT-3 determine the overall level of inhibition in the retina or the amount of inhibitory signal at specific synapses. Mainly interacting with $GABA_C$ receptors, GAT-1 contributes to the shaping of ganglion cell responses, and interacting with $GABA_B$ receptors, this transporter is likely to play important developmental functions. Other important functional actions are exerted through GABA release mediated by GAT-1 reversed transport. These include GABA release by cholinergic/GABAergic starburst amacrine cells and GABA release during early retinal development.

7. Anti-epileptic drugs, retinal GABA and GAT-1

Anti-epileptic drugs have been developed with the aim of contrasting excess excitation by increasing GABAergic inhibition in the central nervous system. Two recently developed compounds, vigabatrin and tiagabin, increase GABA availability through two different mechanisms. Vigabatrin attenuates GABA metabolism by inhibiting the enzyme GABA-transaminase, whereas tiagabine blocks GABA uptake by GAT-1. As

part of the central nervous system, the retina is also affected by treatment with these molecules. Vigabatrin accumulates in the retina and induces a greater increase of GABA concentrations in the retina than in the brain. These abnormally high GABA levels in the retina often result in significant visual field constriction. In contrast, tiagabine does not accumulate in the retina, it does not over-inhibits GAT-1 and does not cause visual field disturbances (102). These observations indicate that interventions on the GABAergic system with anti-epileptic purposes should privilege the compounds, like tiagabine, directed at inhibiting GABA uptake, since these compounds do not seem to significantly impair visual function.

References

1. Kanner BI, Schuldiner S. Mechanism of transport and storage of neurotransmitters. CRC Crit Rev Biochem 1987;22:1-38.

2. Chen NH, Reith ME, Quick MW. Synaptic uptake and beyond: the sodium- and chloride-dependent neurotransmitter transporter family SLC6. Pflugers Arch 2004;447:519-31.

3. Gether U, Andersen PH, Larsson OM, Schousboe A. Neurotransmitter transporters: molecular function of important drug targets. Trends Pharmacol Sci 2006;27:375-83.

4. Deken SL, Beckman ML, Boos L, Quick MW. Transport rates of GABA transporters: regulation by the N-terminal domain and syntaxin 1A. Nat Neurosci 2000;3:998-1003.

5. Attwell D, Barbour B, Szatkowski M. Nonvesicular release of neurotransmitter. Neuron 1993;11:401-7.

6. Bonanno G, Raiteri L, Paluzzi S, Zappettini S, Usai C, Raiteri M. Co-existence of GABA and Glu transporters in the central nervous system. Curr Top Med Chem 2006;6:979-88.

7. Jursky F, Tamura S, Tamura A, Mandiyan S, Nelson H, Nelson N. Structure, function and brain localization of neurotransmitter transporters. J Exp Biol 1994;196:283-95.

8. Worrall DM, Williams DC. Sodium ion-dependent transporters for neurotransmitters: a review of recent developments. Biochem J 1994;297 (Pt 3):425-36.

9. Cammack JN, Rakhilin SV, Schwartz EA. A GABA transporter operates asymmetrically and with variable stoichiometry. Neuron 1994;13:949-60.

10. Quick MW. Substrates regulate gamma-aminobutyric acid transporters in a syntaxin 1A-dependent manner. Proc Natl Acad Sci U S A 2002;99:5686-91.

11. Law RM, Stafford A, Quick MW. Functional regulation of gamma-aminobutyric acid transporters by direct tyrosine phosphorylation. J Biol Chem 2000;275:23986-91.

12. Herbison AE, Augood SJ, Simonian SX, Chapman C. Regulation of GABA transporter activity and mRNA expression by estrogen in rat preoptic area. J Neurosci 1995;15:8302-9.

13. Beckman ML, Bernstein EM, Quick MW. Multiple G protein-coupled receptors initiate protein kinase C redistribution of GABA transporters in hippocampal neurons. J Neurosci 1999;19:RC9.

14. Borden LA. GABA transporter heterogeneity: pharmacology and cellular localization. Neurochem Int 1996;29:335-56.

15. Sarup A, Larsson OM, Schousboe A. GABA transporters and GABA-transaminase as drug targets. Curr Drug Targets CNS Neurol Disord 2003;2:269-77.

16. Cherubini E, Conti F. Generating diversity at GABAergic synapses. Trends Neurosci 2001;24:155-62.

17. Dalby NO. Inhibition of gamma-aminobutyric acid uptake: anatomy, physiology and effects against epileptic seizures. Eur J Pharmacol 2003;479:127-37.

18. Liu QR, Lopez-Corcuera B, Mandiyan S, Nelson H, Nelson N. Molecular characterization of four pharmacologically distinct gamma-aminobutyric acid transporters in mouse brain [corrected]. J Biol Chem 1993;268:2106-12.

19. Borden LA, Murali Dhar TG, Smith KE, Weinshank RL, Branchek TA, Gluchowski C. Tiagabine, SK&F 89976-A, CI-966, and NNC-711 are selective for the cloned GABA transporter GAT-1. Eur J Pharmacol 1994;269:219-24.

20. Yang XL. Characterization of receptors for glutamate and GABA in retinal neurons. Prog Neurobiol 2004;73:127-50.

21. McMahon MJ, Packer OS, Dacey DM. The classical receptive field surround of primate parasol ganglion cells is mediated primarily by a non-GABAergic pathway. J Neurosci 2004;24:3736-45.

22. Kamermans M, Fahrenfort I, Schultz K, Janssen-Bienhold U, Sjoerdsma T, Weiler R. Hemichannel-mediated inhibition in the outer retina. Science 2001;292:1178-80.

23. Verweij J, Hornstein EP, Schnapf JL. Surround antagonism in macaque cone photoreceptors. J Neurosci 2003;23:10249-57.

24. Cook PB, McReynolds JS. Lateral inhibition in the inner retina is important for spatial tuning of ganglion cells. Nat Neurosci 1998;1:714-9.

25. Flores-Herr N, Protti DA, Wassle H. Synaptic currents generating the inhibitory surround of ganglion cells in the mammalian retina. J Neurosci 2001;21:4852-63.

26. Dong CJ, Werblin FS. Temporal contrast enhancement via GABAC feedback at bipolar terminals in the tiger salamander retina. J Neurophysiol 1998;79:2171-80.

27. Caldwell JH, Daw NW, Wyatt HJ. Effects of picrotoxin and strychnine on rabbit retinal ganglion cells: lateral interactions for cells with more complex receptive fields. J Physiol 1978;276:277-98.

28. Lukasiewicz PD, Eggers ED, Sagdullaev BT, McCall MA. GABAC receptor-mediated inhibition in the retina. Vision Res 2004;44:3289-96.

29. Marc RE. Retinal neurotransmitters. In: Chalupa LM, Werner JS, eds. The visual neurosciences. Cambridge (MA): The MIT Press; 2003:304-19.

30. Slaughter MM. Inhibition in the retina. In: Chalupa LM, Werner JS, eds. The visual neurosciences. Cambridge (MA): The MIT Press; 2003:355-68.

31. Casini G, Rickman DW, Brecha NC. Expression of the gamma-aminobutyric acid (GABA) plasma membrane transporter-1 in monkey and human retina. Invest Ophthalmol Vis Sci 2006;47:1682-90.

32. Strettoi E, Masland RH. The number of unidentified amacrine cells in the mammalian retina. Proc Natl Acad Sci U S A 1996;93:14906-11.

33. Casini G, Brecha NC. Colocalization of vasoactive intestinal polypeptide and GABA immunoreactivities in a population of wide-field amacrine cells in the rabbit retina. Vis Neurosci 1992;8:373-8.

34. Marc RE. Structural organization of GABAergic circuitry in ectotherm retinas. Prog Brain Res 1992;90:61-92.

35. Marc RE, Jones BW. Molecular phenotyping of retinal ganglion cells. J Neurosci 2002;22:413-27.

36. Yazulla S. GABAergic mechanisms in the retina. In: Osborne NN, Chader GJ, eds. Progress in Retinal Research. Oxford: Pergamon Press; 1986:1-52.

37. Biedermann B, Eberhardt W, Reichelt W. GABA uptake into isolated retinal Muller glial cells of the guinea-pig detected electrophysiologically. Neuroreport 1994;5(4):438-40.

38. Yu BC, Watt CB, Lam DM, Fry KR. GABAergic ganglion cells in the rabbit retina. Brain Res 1988;439:376-82.

39. Yazulla S, Brecha N. Binding and uptake of the GABA analogue, 3H-muscimol, in the retinas of goldfish and chicken. Invest Ophthalmol Vis Sci 1980;19:1415-26.

40. Pourcho RG, Goebel DJ. Neuronal subpopulations in cat retina which accumulate the GABA agonist, (3H)muscimol: a combined Golgi and autoradiographic study. J Comp Neurol 1983;219:25-35.

41. Massey SC, Blankenship K, Mills SL. Cholinergic amacrine cells in the rabbit retina accumulate muscimol. Vis Neurosci 1991;6:113-7.

42. Hendrickson A, Ryan M, Noble B, Wu JY. Colocalization of [3H]muscimol and antisera to GABA and glutamic acid decarboxylase within the same neurons in monkey retina. Brain Res 1985;348:391-6.

43. Chun MH, Wassle H, Brecha N. Colocalization of [3H]muscimol uptake and choline acetyltransferase immunoreactivity in amacrine cells of the cat retina. Neurosci Lett 1988;94:259-63.

44. Pow DV, Baldridge W, Crook DK. Activity-dependent transport of GABA analogues into specific cell types demonstrated at high resolution using a novel immunocytochemical strategy. Neuroscience 1996;73:1129-43.

45. Brandon C, Lam DM, Wu JY. The gamma-aminobutyric acid system in rabbit retina: localization by immunocytochemistry and autoradiography. Proc Natl Acad Sci U S A 1979;76:3557-61.

46. Agardh E, Ehinger B. Retinal GABA neuron labelling with [3H]isoguvacine in different species. Exp Eye Res 1983;36:215-29.

47. Pourcho RG. Uptake of [3H]glycine and [3H]GABA by amacrine cells in the cat retina. Brain Res 1980;198:33-46.

48. Freed MA, Nakamura Y, Sterling P. Four types of amacrine in the cat retina that accumulate GABA. J Comp Neurol 1983;219:295-304.

49. Borden LA, Smith KE, Hartig PR, Branchek TA, Weinshank RL. Molecular heterogeneity of the gamma-aminobutyric acid (GABA) transport system. Cloning of two novel high affinity GABA transporters from rat brain. J Biol Chem 1992;267:21098-104.

50. Brecha NC, Weigmann C. Expression of GAT-1, a high-affinity gamma-aminobutyric acid plasma membrane transporter in the rat retina. J Comp Neurol 1994;345:602-11.

51. Howd AG, Rattray M, Butt AM. Expression of GABA transporter mRNAs in the developing and adult rat optic nerve. Neurosci Lett 1997;235:98-100.

52. Honda S, Yamamoto M, Saito N. Immunocytochemical localization of three subtypes of GABA transporter in rat retina. Brain Res Mol Brain Res 1995;33:319-25.

53. Johnson J, Chen TK, Rickman DW, Evans C, Brecha NC. Multiple gamma-Aminobutyric acid plasma membrane transporters (GAT-1, GAT-2, GAT-3) in the rat retina. J Comp Neurol 1996;375:212-24.

54. Biedermann B, Bringmann A, Reichenbach A. High-affinity GABA uptake in retinal glial (Muller) cells of the guinea pig: electrophysiological characterization, immunohistochemical localization, and modeling of efficiency. Glia 2002;39:217-28.

55. Sivakami S, Ganapathy V, Leibach FH, Miyamoto Y. The gamma-aminobutyric acid transporter and its interaction with taurine in the apical membrane of the bovine retinal pigment epithelium. Biochem J 1992;283 (Pt 2):391-7.

56. Durkin MM, Smith KE, Borden LA, Weinshank RL, Branchek TA, Gustafson EL. Localization of messenger RNAs encoding three GABA transporters in rat brain: an in situ hybridization study. Brain Res Mol Brain Res 1995;33:7-21.

57. Hu M, Bruun A, Ehinger B. Expression of GABA transporter subtypes (GAT1, GAT3) in the adult rabbit retina. Acta Ophthalmol Scand 1999;77:255-60.

58. Guastella J, Nelson N, Nelson H, et al. Cloning and expression of a rat brain GABA transporter. Science 1990;249:1303-6.

59. Qian X, Malchow RP, O'Brien J, al-Ubaidi MR. Isolation and characterization of a skate retinal GABA transporter cDNA. Mol Vis 1998;4:6.

60. do Nascimento JL, Ventura AL, Paes de Carvalho R. Veratridine- and glutamate-induced release of [3H]-GABA from cultured chick retina cells: possible involvement of a GAT-1-like subtype of GABA transporter. Brain Res 1998;798:217-22.

61. Wassle H, Boycott BB. Functional architecture of the mammalian retina. Physiol Rev 1991;71(2):447-80.

62. Yang CY, Brecha NC, Tsao E. Immunocytochemical localization of gamma-aminobutyric acid plasma membrane transporters in the tiger salamander retina. J Comp Neurol 1997;389:117-26.

63. Ekstrom P, Anzelius M. GABA and GABA-transporter (GAT-1) immunoreactivities in the retina of the salmon (Salmo salar L.). Brain Res 1998;812:179-85.

64. Fletcher EL, Clark MJ, Furness JB. Neuronal and glial localization of GABA transporter immunoreactivity in the myenteric plexus. Cell Tissue Res 2002;308:339-46.

65. Ruiz M, Egal H, Sarthy V, Qian X, Sarkar HK. Cloning, expression, and localization of a mouse retinal gamma-aminobutyric acid transporter. Invest Ophthalmol Vis Sci 1994;35:4039-48.

66. Sinclair JR, Nirenberg S. Characterization of neuropeptide Y-expressing cells in the mouse retina using immunohistochemical and transgenic techniques. J Comp Neurol 2001;432:296-306.

67. Dmitrieva NA, Lindstrom JM, Keyser KT. The relationship between GABA-containing cells and the cholinergic circuitry in the rabbit retina. Vis Neurosci 2001;18:93-100.

68. Jones EM. Na(+)- and Cl(-)-dependent neurotransmitter transporters in bovine retina: identification and localization by in situ hybridization histochemistry. Vis Neurosci 1995;12(6):1135-42.

69. Wilson JR, Cowey A, Somogy P. GABA immunopositive axons in the optic nerve and optic tract of macaque monkeys. Vision Res 1996;36(10):1357-63.

70. Koontz MA, Hendrickson LE, Brace ST, Hendrickson AE. Immunocytochemical localization of GABA and glycine in amacrine and displaced amacrine cells of macaque monkey retina. Vision Res 1993;33(18):2617-28.

71. da Costa BL, Hokoc JN, Pinaud RR, Gattass R. GABAergic retinocollicular projection in the New World monkey Cebus apella. Neuroreport 1997;8(8):1797-802.

72. Crooks J, Kolb H. Localization of GABA, glycine, glutamate and tyrosine hydroxylase in the human retina. J Comp Neurol 1992;315(3):287-302.

73. Minelli A, Brecha NC, Karschin C, DeBiasi S, Conti F. GAT-1, a high-affinity GABA plasma membrane transporter, is localized to neurons and astroglia in the cerebral cortex. J Neurosci 1995;15:7734-46.

74. Ribak CE, Tong WM, Brecha NC. GABA plasma membrane transporters, GAT-1 and GAT-3, display different distributions in the rat hippocampus. J Comp Neurol 1996;367:595-606.

75. Hu M, Bruun A, Ehinger B. Expression of GABA transporter subtypes (GAT1, GAT3) in the developing rabbit retina. Acta Ophthalmol Scand 1999;77:261-5.

76. Calaza Kda C, de Mello MC, de Mello FG, Gardino PF. Local differences in GABA release induced by excitatory amino acids during retina development: selective activation of NMDA receptors by aspartate in the inner retina. Neurochem Res 2003;28:1475-85.

77. Klooster J, Nunes Cardozo B, Yazulla S, Kamermans M. Postsynaptic localization of gamma-aminobutyric acid transporters and receptors in the outer plexiform layer of the goldfish retina: An ultrastructural study. J Comp Neurol 2004;474:58-74.

78. Birnbaum AD, Rohde SK, Qian H, Al-Ubaidi MR, Caldwell JH, Malchow RP. Cloning, immunolocalization, and functional expression of a GABA transporter from the retina of the skate. Vis Neurosci 2005;22:211-23.

79. Zhao JW, Du JL, Li JS, Yang XL. Expression of GABA transporters on bullfrog retinal Muller cells. Glia 2000;31:104-17.

80. Itouji A, Sakai N, Tanaka C, Saito N. Neuronal and glial localization of two GABA transporters (GAT1 and GAT3) in the rat cerebellum. Brain Res Mol Brain Res 1996;37(1-2):309-16.

81. Palacin M, Estevez R, Bertran J, Zorzano A. Molecular biology of mammalian plasma membrane amino acid transporters. Physiol Rev 1998;78:969-1054.

82. Crook DK, Pow DV. Analysis of the distribution of glycine and GABA in amacrine cells of the developing rabbit retina: a comparison with the ontogeny of a functional GABA transport system in retinal neurons. Vis Neurosci 1997;14:751-63.

83. Yang CY. gamma-aminobutyric acid transporter-mediated current from bipolar cells in tiger salamander retinal slices. Vision Res 1998;38:2521-6.

84. Kamermans M, Werblin F. GABA-mediated positive autofeedback loop controls horizontal cell kinetics in tiger salamander retina. J Neurosci 1992;12:2451-63.

85. Malchow RP, Andersen KA. GABA transporter function in the horizontal cells of the skate. Prog Brain Res 2001;131:267-75.

86. Schwartz EA. Depolarization without calcium can release gamma-aminobutyric acid from a retinal neuron. Science 1987;238:350-5.

87. Takahashi K, Miyoshi S, Kaneko A, Copenhagen DR. Actions of nipecotic acid and SKF89976A on GABA transporter in cone-driven horizontal cells dissociated from the catfish retina. Jpn J Physiol 1995;45:457-73.

88. Yang XL, Gao F, Wu SM. Modulation of horizontal cell function by GABA(A) and GABA(C) receptors in dark- and light-adapted tiger salamander retina. Vis Neurosci 1999;16:967-79.

89. Verweij J, Kamermans M, Negishi K, Spekreijse H. GABA sensitivity of spectrally classified horizontal cells in goldfish retina. Vis Neurosci 1998;15:77-86.

90. Haugh-Scheidt L, Malchow RP, Ripps H. GABA transport and calcium dynamics in horizontal cells from the skate retina. J Physiol 1995;488 (Pt 3):565-76.

91. Cammack JN, Schwartz EA. Ions required for the electrogenic transport of GABA by horizontal cells of the catfish retina. J Physiol 1993;472:81-102.

92. Dong CJ, Picaud SA, Werblin FS. GABA transporters and GABAC-like receptors on catfish cone- but not rod-driven horizontal cells. J Neurosci 1994;14:2648-58.

93. Nguyen-Legros J, Versaux-Botteri C, Savy C. Dopaminergic and GABAergic retinal cell populations in mammals. Microsc Res Tech 1997;36(1):26-42.

94. Isaacson JS, Solis JM, Nicoll RA. Local and diffuse synaptic actions of GABA in the hippocampus. Neuron 1993;10:165-75.

95. O'Malley DM, Sandell JH, Masland RH. Co-release of acetylcholine and GABA by the starburst amacrine cells. J Neurosci 1992;12:1394-408.

96. Hull C, Li GL, von Gersdorff H. GABA transporters regulate a standing GABAC receptor-mediated current at a retinal presynaptic terminal. J Neurosci 2006;26:6979-84.

97. Ichinose T, Lukasiewicz PD. GABA transporters regulate inhibition in the retina by limiting GABA(C) receptor activation. J Neurosci 2002;22:3285-92.

98. Hanitzsch R, Kuppers L, Flade A. The effect of GABA and the GABA-uptake-blocker NO-711 on the b-wave of the ERG and the responses of horizontal cells to light. Graefes Arch Clin Exp Ophthalmol 2004;242:784-91.

99. Sandell JH. GABA as a developmental signal in the inner retina and optic nerve. Perspect Dev Neurobiol 1998;5:269-78.

100. Firth SI, Wang CT, Feller MB. Retinal waves: mechanisms and function in visual system development. Cell Calcium 2005;37(5):425-32.

101. Catsicas M, Mobbs P. GABAb receptors regulate chick retinal calcium waves. J Neurosci 2001;21:897-910.

102. Sills GJ. Pre-clinical studies with the GABAergic compounds vigabatrin and tiagabine. Epileptic Disord 2003;5:51-6.

Table 1 – Nomenclature and principal characteristics of GATs

Mouse nomenclature	Rat / Human nomenclature	Characteristic inhibitors	Localization in the central nervous system
GAT1	GAT-1	ACHC, tiagabine	Axon terminals; a few astrocytic processes
GAT2	BGT-1	betaine	Astrocytes
GAT3	GAT-2	β-alanine	Neuronal and non-neuronal elements (low expression)
GAT4	GAT-3	β-alanine	Distal astrocytic processes

Figure legends

Figure 1. Schematic representation of the vertebrate retina. Visual information passes from photoreceptors to bipolar cells (BC) through synapses in the outer plexiform layer (OPL). Horizontal cells provide inhibitory regulation of this flow, although there is uncertainty about the involvement of GABA. In the inner plexiform layer (IPL), bipolar cell axonal terminals contact ganglion cell (GC) dendrites. Here, prominent inhibitory actions provided by GABAergic amacrine cells (AC) importantly contribute to the physiological responses of ganglion cells, whose axons reach the retino-recipient nuclei in the brain. Other abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; NFL, nerve fiber layer; ONL, outer nuclear layer; POS, photoreceptor outer segments.

Figure 2. Pattern of GAT-1 immunoreactivity in the mouse retina. Immunofluorescent somata are mainly amacrine cells located in the proximal INL. Processes of GAT-1 immunolabeled interplexiform cells arise from the immunoreactive fiber plexus in the IPL or from immunostained cell bodies in the INL (arrows) and arborize in the OPL. Displaced amacrine cells in the GCL can also be seen. GAT-1 immunoreactive processes are densely distributed in the IPL, with heavier labeling in the distal and in the proximal part of the layer. Paraformaldehyde-fixed cryostat section (10 μm thick). Immunofluorescence obtained with a rabbit primary antibody directed to GAT-1 (Chemicon) and a secondary goat anti-rabbit antibody conjugated with Alexa Fluor 488 (Molecular Probes). Abbreviations as in figure 1. Calibration bar, 50 μm.

Figure 3. Pattern of GAT-1 and GABA colocalization in the rabbit retina. A, GAT-1 immunostaining; B, GABA immunostaining; C, overlay image of A and B. Note that some GAT-1-expressing amacrine cells are also GABA-immunolabeled (arrows), while there are GABA-containing somata which do not express GAT-1 (asterisks). A GAT-1-expressing amacrine cell which does not contain GABA is also visible (arrowhead). GAT-1 immunostaining as in figure 2; the GABA antibody (Sigma) was made in mouse and visualized with goat anti-mouse secondary antibody conjugated with Alexa Fluor 546 (Molecular probes). Abbreviations as in figure 1. Calibration bar, 20 μm.

Figure 4. GAT-1 and parvalbumin colocalization in the rabbit retina. A, GAT-1 immunostaining; B, parvalbumin immunostaining; C, overlay image of A and B. Parvalbumin antibodies label two different populations of amacrine cells in the rabbit retina, including a class of unidentified GABA-containing amacrine cells and the population of glycinergic AII amacrine cells. Here, two cells with the typical morphology of AII amacrine cells can be seen to display GAT-1 immunoreactivity at or near their plasma membrane (arrows). The parvalbumin antibody (Sigma) was made in mouse and visualized with goat anti-mouse secondary antibody conjugated with Alexa Fluor 546. Abbreviations as in figure 1. Calibration bar, 20 μm.

Figure 5. GAT-1 and VIP colocalization in the rabbit retina. A, GAT-1 immunostaining; B, VIP immunostaining; C, overlay image of A and B. All the VIP-containing

amacrine cells also express GAT-1 immunoreactivity (arrow). The VIP antibody (from H. Wong and Dr. J.H. Walsh, UCLA) was made in mouse and visualized with goat anti-mouse secondary antibody conjugated with Alexa Fluor 546. Abbreviations as in figure 1. Calibration bar, 20 μm.

Figure 6. GAT-1 and CaBP colocalization in the rabbit retina. A, GAT-1 immunostaining; B, CaBP immunostaining; C, overlay image of A and B. None of the CaBP-immunostained horizontal cells, near the OPL, display GAT-1 immunoreactivity. In contrast, some of the CaBP-containing amacrine cells also express GAT-1 (arrow). The CaBP antibody (Sigma) was made in mouse and visualized with goat anti-mouse secondary antibody conjugated with Alexa Fluor 546. Abbreviations as in figure 1. Calibration bar, 20 μm.

Figure 7. Diagram summarizing the composition of the population of GAT-1 immunoreactive amacrine cells in the rabbit retina. 43% of these cells are unidentified GABA-containing amacrine cells, while 38% is represented by identified subsets of GABA-containing amacrine cells, including all indoleamine-accumulating and VIP-containing amacrine cells, and some of the substance P (SP)-containing amacrine cells. In addition, 15% of the GAT-1-immunoreactive amacrine cells contain PVA immunoreactivity and are presumably GABAergic as well. Finally, 4% of the GAT-1 immunoreactive amacrine cells are likely to be AII amacrine cells.

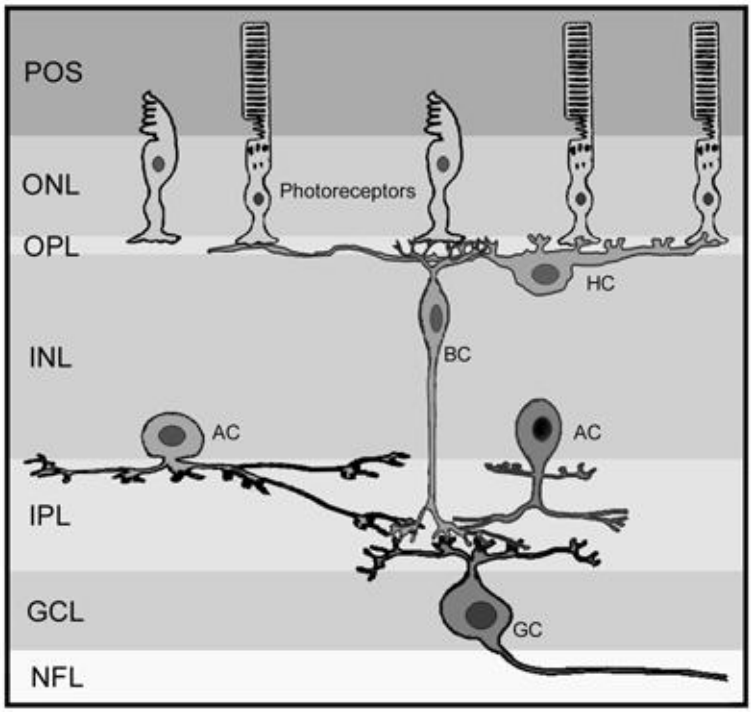
POS
ONL
OPL
INL
IPL
GCL
NFL
Photoreceptors
HC
BC
AC
AC
GC

Fig.1

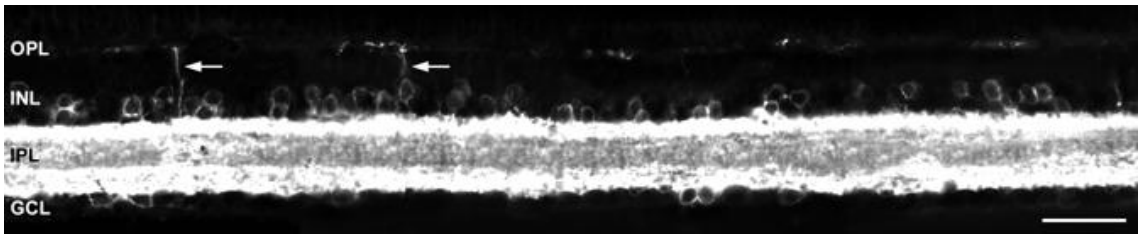
OPL
INL
IPL
GCL

Fig.2

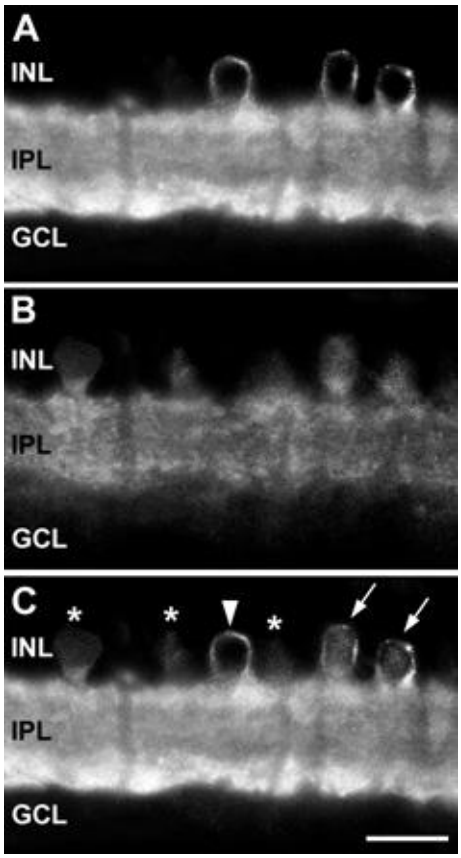

Fig.3

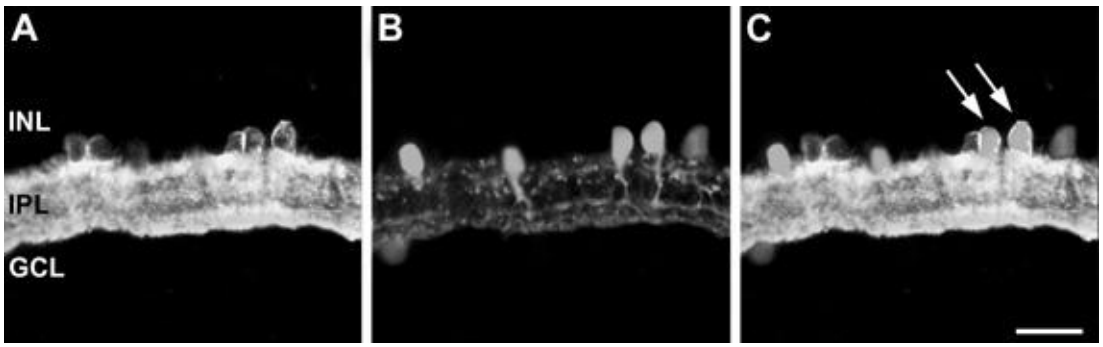

Fig.4

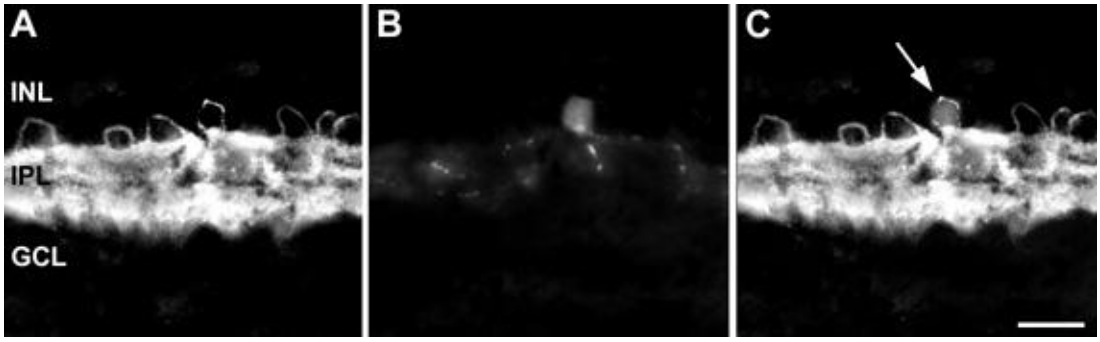

Fig.5

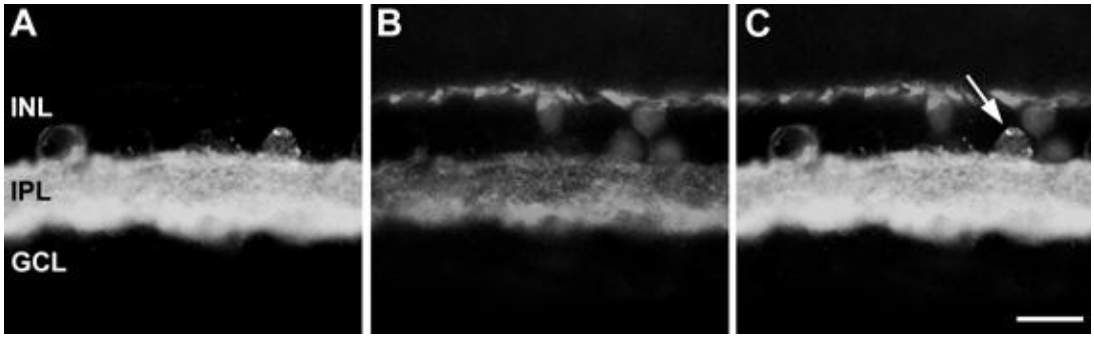

Fig.6

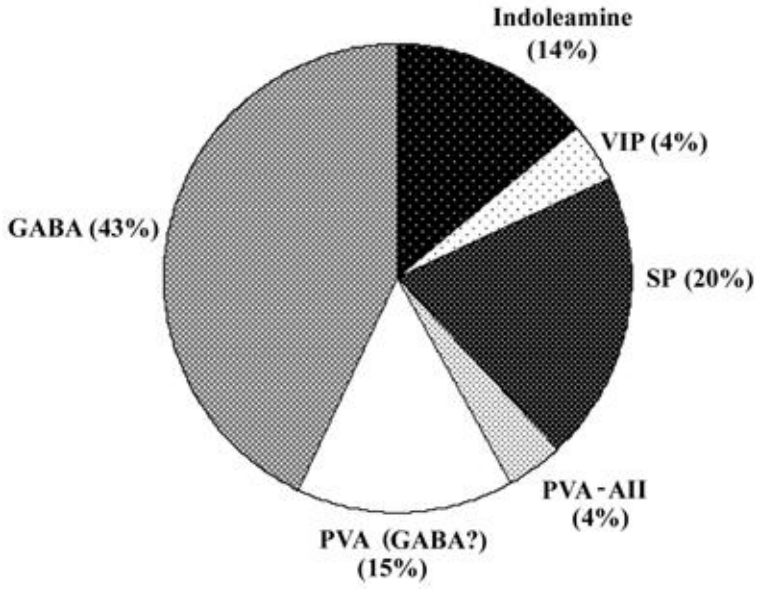

Fig.7